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ART UNIT	PAPER NUMBER
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/403,690	PFEFFER, KLAUS	
	Examiner	Art Unit	
	Juliet Einsmann	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 March 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *21-40* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *21-31 and 33-40* is/are rejected.

7)☒ Claim(s) *32* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *20* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. This action is written in response applicant's correspondence submitted 2/28/02, paper number 19. All previously pending claims have been cancelled, and claims 21-40 have been added. Claims 21-40 are pending. Applicant's amendments and arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections not reiterated in this action have been withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action. **This action is FINAL.**

Information Disclosure Statement

2. The Information Disclosure Statement filed 3/14/02 has been considered, and a signed copy of the 1449 is attached hereto. The reference to the BLAST search has been lined through because it does not indicate a date that the search was performed, or precisely what was searched. The Savarino *et al.* reference itself was not searched, as is indicated on the 1449. If applicant wishes for a record of this search to appear on the front page of any eventually allowed patent, a proper citation of the search should be provided on a 1449.

Claim Rejections - 35 USC § 112, 2nd Paragraph

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 21-29 and 36-40 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 21-29 and 37-40 are indefinite because the preamble of claim 1 recites "A Polymerase Chain Reaction (PCR) method for detection and differentiation of pathogenic

enterobacteria in a sample," but the claim does not contain any process steps which require PCR. The claims require a step of "subjecting said sample and said set of primer pairs to an amplification process," but never particularly require a PCR step as set forth in the preamble. Thus, it is not clear if the claims are meant to require a PCR step or any amplification step that would utilize oligonucleotide primers. Furthermore, the claim never specifically sets forth a method step in which a pathogenic enterobacteria is detected in a sample. The final process step of claim 21 merely sets forth the detection of "amplified product" but does not set forth the relationship between the amplified product and the preamble of the claim which recites that the method is for the detection and differentiation of enterobacteria.

Claims 24-28 and 38 are further indefinite over the recitation of "further comprising an oligonucleotide probe..." because the claim never sets forth a method step that utilizes or employs the probe, thus it is not clear what it means that the method "comprises" the probe. Furthermore, claim 24 recites that the method comprises only a single probe, yet claim 21 requires, at the very least, the amplification of at least two DNA sequences, each specific to a different subgroup of *E. coli*. Therefore, it is unclear if claim 24 intends for only one probe to be included in the method or more than one probe. Claims 25-28 and 38 are indefinite for these same reasons because they depend from claim 24 and do not remedy the cited deficiencies of claim 24.

Claim 26 indefinite because it appears to require that the same labeled oligonucleotide probe be specific for the detection of nine different characteristics, since each section of the claim recites "the labeled oligonucleotide probe" and there is no designation in the claim that these probes are meant to be alternatives. Furthermore, it is noted that none of the previous

claims require the amplification of target DNA for each and every one of the "characteristics" listed in claim 26. Claim 27 is indefinite insomuch as it depends from claim 26 and it is not clear how many or which probes are required for use in the recited methods.

In claim 28, the recitation "fluorescent reporter dye" lacks proper antecedent basis because neither claim 21 nor claim 24 from which claim 28 depends specifically recites a "fluorescent **reporter** dye."

Claims 36 is indefinite over the recitation of "is included in said set of oligonucleotide probes" in line 9 of the claim because the previous description in the claim is directed to the set of oligonucleotide primers, and thus the recitation of "in said set of oligonucleotide probes" is confusing. Claim 36 is further confusing because line 9 recites "and a set of oligonucleotide probes." This recitation is confusing because the first line of the claim recites "A set of primer pairs and set of oligonucleotide primer probes" and it is not clear if the set of "oligonucleotide probes" recited in line 9 of the claim is the set of oligonucleotide primer probes recited in the preamble or a different set of oligonucleotide probes.

Claim Rejections - 35 USC § 102

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

6. Claims 21 and 22 are rejected under 35 U.S.C. 102(b) as being anticipated by Lang *et al.* (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149).

Lang *et al.* teach a PCR method for detection and differentiation of pathogenic enterobacteria in a sample, said method comprising:

adding a set of oligonucleotide primer pairs to said sample, wherein said set of oligonucleotide primers comprises at least one oligonucleotide primer capable of specifically amplifying a DNA sequence of a virulence factor/toxin gene characteristic of enterotoxigenic or enterohemorrhagic E. coli strains;

subjecting said sample and said set of primer pairs to an amplification process to produce amplified product; and

detecting the presence of amplified product (p. 3146, bottom Col. 1-Col. 2).

Lang *et al.* utilize a set of oligonucleotide primer pairs that comprises primers that hybridize to LT (heat-labile toxin gene) of enterotoxigenic E. coli as well as the sltI and sltII genes of enterohemorrhagic E. coli.

7. Claim 13 is rejected under 35 U.S.C. 102(b) as being anticipated by Levine *et al.* (American Journal of Epidemiology (1993 Nov 15) 138(10)849-869).

Levine *et al.* teach a set of probes for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic, enteroinvasive, enteropathogenic, enterohemorrhagic, and enteroaggregative *E. coli* in a sample, wherein for each subgroup of pathogenic *E. coli* a different probe is included in the set. The set of probes taught by probes for the detection of the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC (see p. 854).

Claim Rejections - 35 USC § 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. Claims 21, 22, 23, and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lang *et al.* (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149) in view of Yamamoto *et al.* (Journal of Bacteriology, Aug. 1983, Vol. 155, No. 2, p. 728-733) and further in view of Hogan *et al.* (US 5595874).

Lang *et al.* teach a PCR method for detection and differentiation of pathogenic enterobacteria in a sample, said method comprising:

adding a set of oligonucleotide primer pairs to said sample, wherein said set of oligonucleotide primers comprises at least one oligonucleotide primer capable of specifically amplifying a DNA sequence of a virulence factor/toxin gene characteristic of enterotoxigenic or enterohemorrhagic E. coli strains;

subjecting said sample and said set of primer pairs to an amplification process to produce amplified product; and

detecting the presence of amplified product (p. 3146, bottom Col. 1-Col. 2).

Lang *et al.* utilize a set of oligonucleotide primer pairs that comprises primers that hybridize to LT (heat-labile toxin gene) of enterotoxigenic E. coli as well as the sltI and sltII genes of enterohemorrhagic E. coli.

Lang *et al.* do not teach methods or sets of primers and probes which include instant SEQ ID NO: 1, 2, or 19.

Yamamoto *et al.* teach the nucleotide sequence of heat labile toxin of E. coli pathogenic for humans (Figure 2). Instant SEQ ID NO: 1 consists of nucleotides 49-70 of this sequence, instant SEQ ID NO: 2 consists of the complement of nucleotides 367-388 of this sequence, and instant SEQ ID NO: 19 consists of nucleotides 78-104 of the sequence taught by Yamamoto *et al.* on page 730.

Hogan et al. (US 5595874) teach the use of specific primers and furthermore provides specific guidance for the selection of primers,

> "At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.
>
> First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some others). Second, the stability of the probe:target nucleic acid hybrid should be maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10°C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less preferred. Finally, probes with extensive self complementarity should be avoided (Col. 6, line 50-Col. 7, line 13)."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have substituted primers and probes taken from the sequences taught by

Yamamoto *et al.* in the methods taught by Lang *et al.* The ordinary practitioner would have been motivated to make such a substitution in order to have provided a method that is a functional equivalent of the method taught by Lang *et al.* in that both methods would be useful for the detection of enterotoxigenic E. coli. The teachings of Hogan *et al.* would have provided adequate direction to the ordinary practitioner in the selection of the probes and primers from the sequence taught by Yamamoto *et al.* Thus, in view of the combined teachings of Lang *et al.* in view of Yamamoto *et al.* and Hogan *et al.* the instantly rejected claims are prima facie obvious.

With regard to the specific PCR parameters recited in claim 29, these are considered to be parameters obtained by routine optimization of an assay. As noted in *In re Aller*, 105 USPQ 233 at 235, "More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the PCR parameters claimed performed are other than routine, that the methods resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

10. Claims 21, 22, 24, 25, 26 and 28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lang *et al.* (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149) in view of Livak *et al.* (PCR Methods and Applications (1995) 4:357-362).

Lang *et al.* teach a PCR method for detection and differentiation of pathogenic enterobacteria in a sample, said method comprising:

adding a set of oligonucleotide primer pairs to said sample, wherein said set of oligonucleotide primers comprises at least one oligonucleotide primer capable of specifically

amplifying a DNA sequence of a virulence factor/toxin gene characteristic of enterotoxigenic or enterohemorrhagic E. coli strains;

subjecting said sample and said set of primer pairs to an amplification process to produce amplified product; and

detecting the presence of amplified product (p. 3146, bottom Col. 1-Col. 2).

Lang *et al.* utilize a set of oligonucleotide primer pairs that comprises primers that hybridize to LT (heat-labile toxin gene) of enterotoxigenic E. coli as well as the sltI and sltII genes of enterohemorrhagic E. coli.

Lang *et al.* do not teach methods wherein a polymerase having 5'-3' exonuclease activity is used for the amplification of DNA and a probe labeled at both ends is used to detect amplified samples.

Livak *et al.* teach a PCR-based assay that uses the hydrolysis of an internal fluorogenic probe to monitor the amplification of the target (ABSTRACT). The method taught by Livak *et al.* utilizes a polymerase having 5'-3; exonuclease activity and a probe labeled at the 5' end with the fluorescent dye 6-carboxyfluorescein (6-FAM) and also labeled at the 3' end with the fluorescent quencher dye 6-carboxytetramethyl-rhodamine(TAMARA) (Fig. 2). The labeled probe hybridizes with the target DNA and is included in the amplification process.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have combined the methods taught by Levine *et al.* in view of Lang *et al.* with those taught by Livak *et al.* The ordinary practitioner would have been motivated to use an assay such as the one taught by Livak *et al.* for the detection of E. coli since Livak *et al.* teach that such a method is a homogenous assay for detecting the accumulation of specific PCR

products and probes with a label attached to the 5' end and a quencher at the 3' end exhibit a larger signal in the 5' nuclease PCR assay than internally labeled probes (Abstract).

11. Claim 27 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lang *et al.* in view of Livak *et al.* as applied to claims 21, 22, 24, 25, 26 and 28 above, and further in view of both Yamamoto *et al.* and Hogan *et al.*

The teachings of Lang *et al.* in view of Livak *et al.* are applied to claim 27 as they were applied in the rejection of claims 21, 22, 24, 25, 26, and 28.

Lang *et al.* do not teach methods which utilize a probe with the sequence of SEQ ID NO: 19.

Yamamoto *et al.* teach the nucleotide sequence of heat labile toxin of E. coli pathogenic for humans (Figure 2). Instant SEQ ID NO: 1 consists of nucleotides 49-70 of this sequence, instant SEQ ID NO: 2 consists of the complement of nucleotides 367-388 of this sequence, and instant SEQ ID NO: 19 consists of nucleotides 78-104 of the sequence taught by Yamamoto *et al.* on page 730.

Hogan et al. (US 5595874) teach the use of specific primers and furthermore provides specific guidance for the selection of probes,

> "At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.
>
> First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some others). Second, the stability of the probe:target nucleic acid hybrid should be

> maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10°C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less preferred. Finally, probes with extensive self complementarity should be avoided (Col. 6, line 50-Col. 7, line 13)."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have substituted primers and probes taken from the sequences taught by Yamamoto *et al.* in the methods taught by Lang *et al.* in view of Livak *et al.* The ordinary practitioner would have been motivated to make such a substitution in order to have provided a method that is a functional equivalent of the method taught by Lang *et al.* in view of Livak *et al.* in that both methods would be useful for the detection of enterotoxigenic E. coli. The teachings of Hogan *et al.* would have provided adequate direction to the ordinary practitioner in the selection of the probes and primers from the sequence taught by Yamamoto *et al.* Thus, in view of the combined teachings of Lang *et al.* in view of Livak *et al.*, these taken further in view of Yamamoto *et al.* and Hogan *et al.* the instantly rejected claims are prima facie obvious.

12. Claims 30, 33, 36, and 37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* (American Journal of Epidemiology, Nov. 15, 1993, 138(10): 849-869) in view of Lang *et al.* (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149).

Levine *et al.* teach a method for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC),

enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EaggEC) in a sample, wherein the sample is human fecal matter. Levine *et al.* specifically teach the use of oligonucleotide probes to the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC (see p. 854).

Levine *et al.* do not teach a method that utilizes PCR amplification of the target region, thus Levine *et al.* do not teach a set of oligonucleotide primer pairs.

Lang *et al.* teach a method for detection of pathogenic enterobacteria in a sample comprising PCR amplification of DNA isolated from said sample using a set of oligonucleotide primer pairs that allow the differentiation of enterotoxigenic and enterohemorrhagic E. coli strains by amplification of a toxin gene characteristic for the respective group of the pathogenic E. coli strains (p. 3146-3147). The LT (heat-labile) gene was used to detect enterotoxigenic E. coli, and the SLT I and SLT II genes were used to detect enterohemorrhagic E. coli. For use in their methods, Lang *et al.* a set of primers appropriate for their amplification method and a set of labeled probes useful to detect the amplified products (p. 3146). Furthermore, Lang *et al.* include methods in which hybridization probes were used to detect the amplification products (p. 3146).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Levine *et al.* so as to have included a PCR step for the amplification of the target DNA prior to the utilization of the hybridization probes. The ordinary practitioner would have been motivated by the success of Lang *et al.* in developing such a PCR method for the detection of pathogenic enterobacteria, and the ordinary

practitioner would have been motivated to include an amplification step in the methodology taught by Lang *et al.* in order to have provided a larger quantity of target DNA for the probes. Lang *et al.* teach that their PCR method provides the advantage of rapidly detecting E. coli in a sample, and thus the ordinary practitioner would have been further motivated to use such a method. Thus, the claimed sets of oligonucleotide primers and probes would have been prima facie obvious in light of the teachings of Levine *et al.* in view of Lang *et al.*

13. Claim 31 is rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of Lang *et al.* as previously applied to claims 30, 33, 36, and 37, and further in view of Savarino *et al.* (PNAS USA, Vol. 90, pp. 3093-3097 (1993)).

The teachings of Levine *et al.* in view of Lang *et al.* are applied to claim 31 as they are previously applied to claims 30, 33, 36, and 37. Levine *et al.* further teach that the EAggEC plasmid which is the target of one of their probes encodes the EAggEC heat stable enterotoxin (p. 854). However they do not specifically teach what portion of the plasmid is targeted by their probe.

Levine *et al.* in view of Lang *et al.* do not teach a method utilizes a primer set which would hybridize to a gene encoding heat stable toxin of enteroaggregative *E. coli*.

Savarino *et al.* teach that some EAggEC produce a heat stable enterotoxin named EAST1. Savarino *et al.* provide the DNA sequence of the gene encoding the toxin, and they provide a PCR amplification assay for the amplification the gene (p. 3094 and Fig. 2).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have included a primer pair designed to amplify a portion of the EAST1 gene in the detection method taught by Levine *et al.* in view of Lang *et al.* With such an

inclusion, the method taught by Levine *et al.* in view of Lang *et al.* in view of Savarino *et al.* would utilize a set of primers which comprises a pair of primers that hybridizes to a gene encoding heat stable toxin of EaggEC. The ordinary practitioner would have been motivated to have included the additional primer pair set in order to have utilized yet another tool for the detection of pathogenic *E. coli* in a sample, thus providing a more comprehensive assay for the detection of diarrhea causing *E. coli*.

14. Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of both Savarino *et al.* (PNAS USA (1993) 90:3093-3097) and Louie *et al.* (Epidemiol. Infect. (1994), 112:449-461).

Levine *et al.* teach a set of probes for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EaggEC) in a sample, wherein for each subgroup of pathogenic *E. coli* a different probe is included in the set. The set of probes taught by probes for the detection of the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC (see p. 854).

Levine *et al.* do not teach a set of probes comprising a probes specific for heat stable characteristic for EaggEC or the eae gene.

Savarino *et al.* teach that some EAggEC produce a heat stable enterotoxin named EAST1. Savarino *et al.* provide the DNA sequence of the gene encoding the toxin, and they

provide a PCR amplification assay for the amplification the gene (p. 3094 and Fig. 2). Savarino *et al.* further teach the use of the PCR product produced by their method as a probe in southern blot analysis (p. 3094).

Louie *et al.* teach PCR assays and probes that allow the specific identification of EPEC which contain the *eae* gene (Table 2; p. 452-453). Louie *et al.* teach that their methods "will be useful methods for subclassification of EPEC and VTEC (p. 459)."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have included probes to the EaggEC heat stable enterotoxin gene and the eae gene in the probe set taught by Levine *et al.* The ordinary practitioner would have been motivated to provide a set comprising all of these probes in order to have provided a set of probes which is useful for the detection and differentiation of many different types of toxigenic *E. coli*.

15. Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of both Savarino *et al.* (PNAS USA (1993) 90:3093-3097) and Louie *et al.* (Epidemiol. Infect. (1994), 112:449-461) as applied to claim 14 above, and further in view of all of the following: Yamamoto *et al.* (Journal of Bacteriology, Aug. 1983, Vol. 155, No. 2, p. 728-733), Moseley *et al.* (GenBank M34916), Yamamoto *et al.* (Infection and Immunology (1996) 64 (4): 1441-1445), Schmidt *et al.* (Journal of Clinical Microbiology (1995) 33(3): 701-705), Lampel *et al.* (US 5041372), Franke *et al.* (Journal of Clinical Microbiology (1994) 32(10):2460-2463), Kaper (1995, GenBank Accession Z11541), Paton *et al.* (GenBank Z36899), and Paton *et al.* (GenBank L11079).

The teachings of Levine *et al.* in view of Savarino *et al.* and Louie *et al.* are applied to this claim as discussed above in the rejection of claim 14. Levine *et al.* in view of Savarino *et al.* and Louie *et al.* do not provide probes consisting of the specific sequences recited in claim 15.

However, the full length sequences of all of genes from which the probes used in the methods of Levine *et al.*, Savarino and Louie *et al.* were derived were known in the prior art at the time the invention was made.

Instant SEQ ID NO: 19 consists of nucleotides 78-104 of the sequence taught by Yamamoto *et al.* (1983) on page 730.

Instant SEQ ID NO: 20 consists of the complement of nucleotides 306-334 of the sequence taught by Moseley *et al.*

Instant SEQ ID NO: 21 consists of nucleotides 30-69 of the EAST 1 sequence taught by Yamamoto *et al.* (1996) in figure 4.

Instant SEQ ID NO: 22 consists of the complement of nucleotides 639-668 of the pCVD432 plasmid sequence taught in figure 1 of Schmidt *et al.*

Instant SEQ ID NO: 23 consists of the complement of nucleotides 177-202 of the probe taught by Lampel *et al.* (Col. 13-14).

Instant SEQ ID NO: 24 consists of nucleotides 574-601 of the EAF probe taught by Franke *et al.*

Instant SEQ ID NO: 25 consists the complement of nucleotides 908-935 of the sequence for the eae gene disclosed in GenBank Z11541.

Instant SEQ ID NO: 26 consists of the complement of nucleotides 1338-1367 of the sequence taught by Paton *et al.*

The specific probes of the instant invention represent structural and functional homologues of the longer probes taught in the prior art. That is, the probes recited in claim 35 would be expected to detect the larger sequences of which they are fragments, much like the probes taught in Levine *et al.*, Savarino *et al.* and Louie *et al.* In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the court determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologues, however, the court stated

> "Normally, a *prima facie* case of obviousness is based upon structural similarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologues because homologues often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties."

Since the claimed oligonucleotides simply represent structural homologues of the full length disclosed sequences and the probes disclosed, a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed probes are *prima facie* obvious over the cited reference in the absence of secondary considerations.

16. Claims 39 and 40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of Lang *et al.* in view of Tsen *et al.* (Journal of Food Protection (1996) Vol. 59, No. 8, pp. 795-802).

Levine *et al.* teach a method for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EaggEC) in a sample, wherein

the sample is human fecal matter. Levine *et al.* specifically teach the use of oligonucleotide probes to the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC (see p. 854).

Levine *et al.* do not teach a method that utilizes PCR amplification of the target region.

Lang *et al.* teach a method for detection of pathogenic enterobacteria in a sample comprising PCR amplification of DNA isolated from said sample using a set of oligonucleotide primer pairs that allow the differentiation of enterotoxigenic and enterohemorrhagic E. coli strains by amplification of a toxin gene characteristic for the respective group of the pathogenic E. coli strains (p. 3146-3147). The LT (heat-labile) gene was used to detect enterotoxigenic E. coli, and the SLT I and SLT II genes were used to detect enterohemorrhagic E. coli. For use in their methods, Lang *et al.* a set of primers appropriate for their amplification method and a set of labeled probes useful to detect the amplified products (p. 3146). Lang *et al.* further teach that both ETEC and EHEC have been associated with the ingestion of food and are both known to be pathogenic to humans, causing symptoms including diarrhea.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Levine *et al.* so as to have included a PCR step for the amplification of the target DNA prior to the utilization of the hybridization probes. The ordinary practitioner would have been motivated by the success of Lang *et al.* in developing such a PCR method for the detection of pathogenic enterobacteria, and the ordinary practitioner would have been motivated to include an amplification step in the methodology taught by Lang *et al.* in order to have provided a larger quantity of target DNA for the probes.

Lang *et al.* teach that their PCR method provides the advantage of rapidly detecting E. coli in a sample, and thus the ordinary practitioner would have been further motivated to use such a method.

Levine *et al.* in view of Lang *et al.* do not teach methods in which these pathogenic bacteria are detected in consumables.

However, methods for the detection of pathogenic bacteria in consumables such as milk were routine in the art at the time the invention was made. Such a method for the detection of the LT gene of E. coli in milk is exemplified by Tsen *et al.* (ABSTRACT).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have used a method such as the one taught by Lang *et al.* in order to detect the presence of know pathogenic organisms in milk. The ordinary practitioner would have been motivated to create such a method in order to provide a method for the screening milk samples for possible E. coli infections.

Response to Remarks

New rejections have been set forth under 112 2nd, 102(b), and 103, in light of the newly filed claims. Applicant's arguments are addressed insofar as they apply to the new rejections of the claims.

Applicant argues that Levine *et al.* does not meet the limitations of claim 33 because the probes taught by Levine *et al.* are cloned fragments, which applicant argues are not oligonucleotides. However, the examiner fails to see the distinction being made by applicant. The broadest reasonable interpretation of "oligonucleotide" includes any nucleic acid that is an

"oligomer" of nucleotides, that is, nucleic acid probes that have more than one nucleotide attached to one another. Under this interpretation, the probes taught by Levine *et al.* are in fact oligonucleotide probes, regardless of their length or the method of obtaining the probes. A careful review of the claims and specification does not indicate that any further structural limitation has been implied by the use of the word "oligonucleotide" in the claims.

Applicant further points out that Levine *et al.* does not teach PCR amplification of the genes, and thus does not use Real Time PCR as in, for example, claim 33. However, claim 33 is not a method claim, it is a product claim. The recitation "useful for detection and differentiation of pathogenic enterobacteria in a sample by Real-Time PCR" is merely a statement of intended use. A recitation of the intended use of the claimed invention must result in a structural difference between the claimed invention and the prior art in order to patentably distinguish the claimed invention from the prior art. In the instant case, set of probes taught by Levine *et al.* appears to meet the structural limitations of claim 33 (see MPEP 2111.02).

Applicant sets forth arguments concerning the possible rejection of claims 21, 22, 29, 27, and 38 under Levine *et al.* in view of Lang *et al.* However, these claims have not been rejected by this combination of references. Nonetheless, the arguments are addressed.

Applicant sets forth the argument that, absent a teaching by Levine *et al.* of PCR amplification of DNA sequences, Levine *et al.* cannot render the claimed invention obvious. This analysis may have been if Levine *et al.* were used to reject the instantly pending claims alone, however, this is not the case in the instant action. In each combination where Levine *et al.* is used in a 103 to reject primer pairs or amplification methods a secondary reference has been provided to teach PCR amplification for the detection of enterobacteria. To suggest that because

Levine *et al.* does not teach PCR amplification, no possible combination of references with Levine *et al.* in the can obviate the claimed invention is an improper piecemeal analysis. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicant sets forth a similar piecemeal analysis of Lang *et al.* concluding that because Lang *et al.* only teach the amplification of a toxin gene characteristic for one of the two subgroups and not detection and differentiation of all five subgroups of pathogenic E. coli, Lang *et al.* cannot render the claimed invention either alone or in combination. As a first point, it is noted that Lang *et al.* demonstrate the amplification of toxin genes from two of the five subgroups listed in applicant's claim 1. Nevertheless, a blanket statement concerning any possible 103 rejection using Lang *et al.* is an improper piecemeal argument because it does not consider the combinations of references that have been used to reject the claims. Furthermore, in light of applicant's new claims, Lang *et al.* anticipates claims 21 and 22 because these require only that "at least one primer pair" in the amplification reaction be able to specifically amplify a virulence/toxin gene from the five listed subgroups, and do not require at any point the differentiation and/or detection of all five subgroups of E. coli. Thus, applicant's arguments are not relevant to at least claims 21 and 22.

Applicant sets forth arguments that the art "taught away from using a set of oligonucleotide primer pairs in a PCR method to detect and identify the subgroups of pathogenic E.coli." Applicant cites as an example of this teaching away Schmidt *et al.*'s suggestion that

PCR alone is not sufficient for a diagnosis of EaggEC strains. Schmidt *et al.*'s actual quotation is "The PCR technique used here enables a more rapid diagnosis of EAggEC than other techniques currently available. However, the EAggEC probe or the adherence test must still supplement the PCR to identify the disease causing strains (sentence bridging p. 704-705)." In fact, this is not a teaching away from the use of PCR for the identification of the subgroups of pathogenic E. coli, but a clear teaching to do so, albeit in combination with other techniques. Applicant's claims are drawn using the open claim language "comprising" which implies that any method which utilizes the recited steps is encompassed within the claims, even if additional steps are used in the prior art method. Thus the invention to which applicant refers in the arguments "oligonucleotide primers and proves for the detection and differentiation of all known E. coli strains by PCR alone" is not claimed. Furthermore, it is noted that such a claim would require further search and consideration beyond the instantly claimed invention, or any claims which have been previously set forth in the prosecution of this application.

Applicant further argues that Savarino *et al.* teach away from using a set of primer pairs in a PCR method to detect and identify the subgroups of pathogenic E. coli, because one primer in a primer pair used by Savarino *et al.* is not specific for EaggEC. However, this is not a teaching away from the instantly claimed invention. Again, Savarino *et al.* are specifically teaching the use of primer pairs for the detection of E. coli. That one of the two primer pairs used by Savarino *et al.* is not specific only for EaggEC is irrelevant. Savarino *et al.* are using PCR amplification to obtain a particular nucleic acid for sequencing and use as a nucleic acid probe. Savarino *et al.* do not suggest that a method which uses PCR amplification to detect

subgroups of E. coli is not possible or desirable. They do not teach away from the instantly claimed invention.

Applicant's analyses on pages 17-20 of the response address further 103 rejections that were made in the previous office action to address claims that are not longer pending. Thus, these arguments are no longer relevant to the instant rejections as the instant rejections represent new combinations of references necessitated by the newly filed claims. Particular points in these arguments that may be relevant to the instant rejections are addressed herein.

Where a previous rejection relied first on the combination of Levine *et al.* and Lang *et al.* to reject a particular set of claims, applicant relied on the previously set forth arguments as the primary basis of the response (for example the discussion of rejection of claim 3 in page 17). The arguments further describe the deficiencies of each individual cited reference but do not address the combinations of references. This amounts to a piecemeal analysis of the additional references. Applicant is reminded that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references.

Furthermore, applicant again points out that the cited references do not teach or suggest the instantly claimed primers or probes capable of specifically hybridizing and amplifying all five subgroups of pathogenic E. coli. As a first point, the rejected claim being discussed was claim 3, a method claim. The instantly pending claim that is closest in scope and form to claim 3 is instant claim 23. This claim DOES NOT require the use of a set of probes and primers capable of specifically hybridizing and amplifying all five subgroups of pathogenic E. coli. Claim 23 depends from claim 22 which in turn depends from claim 21. Claim 21 requires a step of adding a set of oligonucleotide primer pairs to a sample, wherein the set of primers

"comprises **at least one oligonucleotide** primer pair capable of specifically amplifying a DNA sequence of a virulence factor/toxin gene characteristic for **one** of the subgroups... (emphasis added)." Thus, applicant is setting forth arguments about claims that are not present in the instant application. Such claims, if they were filed, would have a different scope from the instantly pending claims, and would require further search and consideration, and quite possibly would result in the setting forth of different rejections.

Applicant states on page 18 of the arguments that "None of the cited references either alone or in combination disclose the set of specific oligonucleotide probes recited in claim 27." However, applicant provides no reasoning to support this assertion. Claim 27 has been rejected as being prima facie obvious for the reasons set forth in the rejections stated in this office action. It is noted that claim 27 is a method claim that depends from claim 26 and claim 26 has further dependencies that extend to dependency upon claim 21. Claim 27 does not require that the entire set of probe recited in claim 27 be utilized in the method, it merely defines the sequence of the probe that is required in claim 26, which depends from claim 25, which depends from claim 24, which requires only "**an** oligonucleotide probe capable of hybridizing to a DNA sequence of a virulence factor/toxin gene characteristic for **one** of the subgroups...(emphasis added)."

Applicant states on page 19 of the response that two of the four primer pairs in Louie *et al.* do not specifically recognize EPEC. However, this is irrelevant for the rejection of claims 34 and 35 which are product claims that require probes specific for particular genes. Louie *et al.* is relied upon for the teaching of probes specific for the *eae* gene, which are clearly provided within Louie *et al.*, as discussed in the rejection.

Applicant states that new claim 25 corresponds to cancelled claim 15. It appears that applicant is mistaken because cancelled claim 15 is drawn to a set of oligonucleotide probes and new claim 25 is a method claim. Newly added claim 25 is newly rejected as set forth in this office action. The rejection of claim 25 relies on different references from the rejection of cancelled claim 15.

All of the amendments and arguments having been addressed, the instantly pending claims remain rejected for the reasons set forth herein.

Conclusion

17. Claim 32 objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

18. Claim 32 is free of the prior art. Claim 32 requires, among other things, a pair of nucleic acid primers directed to SEQ ID NO: 5 and 6. The prior art does not teach or suggest such a primer pair.

19. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37

CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

20. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Juliet C. Einsmann whose telephone number is (703) 306-5824. The examiner can normally be reached on Monday through Thursday, 7:00 AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, W. Gary Jones can be reached on (703) 308-1152. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 and (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

JEFFREY FREDMAN
PRIMARY EXAMINER

Juliet C. Einsmann
Examiner
Art Unit 1655

May 22, 2002